Untargeted analysis of contaminants in river water samples: comparison between two different sorbents for solid-phase extraction followed by liquid chromatography-high-resolution mass spectrometry determination

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Abstract

In the last years, the employment of high-resolution mass spectrometry (HRMS) is increasing in several analytical applications, especially in the environmental field. Indeed, HRMS allows performing both the suspect screening and the untargeted analysis, particularly suitable for detecting the contaminants of emerging concern and their possible transformation products, including compounds recently introduced in the market and whose analytical standards are not available or whose presence is still unknown.

In this work, we wanted to apply an untargeted approach for the analysis of the main contaminants, which could be present in surface water as the result of ineffective removal by wastewater treatment plants or illicit release. For this reason, first solid-phase extraction conditions for two different sorbents, namely the popular one Oasis HLB and graphitized carbon black, were optimized by two distinct designs of experiments. Then, river water samples collected in the Municipality of Rome (Italy) were analyzed using both sorbents in both electrospray (ESI) ion polarities. The compounds were tentatively identified by matching their tandem mass spectra with the mzCloud mass spectral library and manual validation, resulting in a total of 241 tentatively identified compounds (211 and 40 in positive and negative ESI mode, respectively), mainly belonging to pharmaceuticals.

Keywords

Environmental contaminants; pharmaceuticals; design of experiment; high-resolution mass spectrometry; surface water; solid-phase extraction

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

1. Introduction

Nowadays, likely tens to hundreds of thousands of anthropogenic chemicals are dispersed in the aquatic environment, and only a small fraction is regularly monitored, and even less are regulated [1,2]. These substances belong to very diverse chemical classes, and they are generally referred to as contaminants of emerging concern (CECs). Indeed, the term CECs comprise substances recently introduced into the environment, substances known to be present in the environment, but not recognized before as dangerous to ecosystems, as well as substances being present in the environment for a long time but detected only recently with the improvement of the analytical techniques [3]. CECs are constituted mainly by pharmaceuticals and personal care products (PPCPs), hormones, UV filters, UV stabilizers, pesticides, plasticizers, drugs of abuse, and other industrial chemicals; also, some CECs are recognized as endocrine disruptors. Moreover, CEC transformation products (TPs) should be studied as well since they could be even more toxic than the parent compounds.

At present, more than 3000 active pharmaceutical ingredients and hundreds of active compounds for personal care products are on the market [4]. These chemicals and their metabolites (both produced by human metabolism and biotic and abiotic environmental factors) ultimately end up in domestic wastewater, which is globally retained the major water contamination source whereas emissions from the pharmaceutical industry, agriculture, and aquaculture can be very important locally [5]. Wastewater treatment plants (WWTPs) are not able to completely remove PPCPs [3,6,7], which, therefore, can contaminate surface waters; antibiotics comprise approximately half of PPCP contamination [8], which could contribute to the development of antibiotic resistance [9,10]. In analogy with pharmaceutical compounds, also drugs of abuse are only partially metabolized by human body, therefore both parent compounds and their active metabolites can reach the surface waters by WWTP effluents or direct illicit release [7].

The European Union, in the Directive 2008/105/EC setting environmental quality standards in the field of water policy (amended by Directive 2013/39/EU [11]), deals with the presence in surface water of certain substances or groups of substances (ca. 50). The Directive 2008/105 also established

a watch list (regularly updated every 2 years; the last one is reported in the Commission Decision (EU) 2020/1161 [12]) of substances for which EU-wide monitoring data are to be gathered to support future prioritization exercises. Therefore, it is important to develop efficient strategies to monitor the presence of CECs belonging to different classes in surface waters [2,3,6].

Reversed-phase (RP) liquid chromatography coupled with mass spectrometry (LC-MS) by electrospray (ESI) source is the method of choice for the determination of PPCPs, their metabolites, and TPs in aqueous matrices [13,14]. Operating with low-resolution MS, only targeted analysis is possible; in this case, the number of compounds to monitor is limited by the availability of analytical standards. When the aim is to characterize an environmental water sample, containing a large number of CECs and other substances whose standards are not available or whose presence is only suspected or even unknown, high-resolution mass spectrometry (HRMS) is needed [15]. Furthermore, the development of new PPCPs and the potential for biotic and abiotic transformation of PPCPs requires untargeted techniques to identify features of interest in environmental samples that targeted analyses cannot [3]. In fact, in the last few years, HRMS is representing the technique of choice in environmental monitoring studies [16]. Undoubtedly, the huge technical advances in HRMS instrumentation, its more affordable cost than in the past, as well as the availability of more friendly tools for mass spectra handling and interpretation, and mass spectral libraries development, have allowed many research and monitoring laboratories to use it [13]. Moreover, differently from low-resolution MS-targeted methods, HRMS allows the unique opportunity to perform a retrospective analysis on the previously acquired data [1,17].

For CEC extraction from water samples, solid-phase extraction (SPE) using different sorbents is still the most widely employed strategy. Hydrophilichydrophobic polymeric materials are generally the preferred ones [18–22]In this work, we optimized by Design of Experiments (DoE) two different SPE protocols for recovering 30 target compounds from water samples. The selected sorbents were Oasis HLB and graphitized carbon black (GCB). Then, the optimized conditions were applied to river water samples collected in the area of Rome (Italy), which were subject to HRMS-based untargeted

analysis. In this way, 241 CECs (mainly belonging to pharmaceuticals) were tentatively identified by matching their tandem mass spectra with those contained in the mzCloud (https://www.mzcloud.org/) spectral database.

2. Materials and Methods

2.1. Chemicals and Materials

Solvents used for sample extraction, i.e., HPLC-grade methanol (MeOH) and CH_2Cl_2, were obtained from VWR International (Milan, Italy). Ultra-pure water and acetonitrile (ACN) of LC-MS grade were purchased from Thermo Fisher Scientific (Waltham, MA, USA); LC-MS grade MeOH was provided by Romil Pure Chemistry (Pozzuoli, NA, Italy).

All the other solvents, reagents, and analytical standards, as well as formic acid (FA), trifluoroacetic acid (TFA), ethylenediaminetetraacetic acid disodium salt (EDTA), and tetramethylammonium chloride (TMACl) were provided by Merck Life Science (Milan, Italy).

Analytical standards of the 30 compounds used for method optimization are listed in Supplementary Table S1. They belonged to different chemical classes and were grouped into floroquinolones (3), macrolides (3) penicillins (7), sulfonamides (6), steroid hormones (5), imidazoles (2), and herbicides (3 triazines and 1 benzamide).

Caffeine-(trimethyl-d9) (caffeine-d9) was used as volumetric internal standard (IS). Individual stock standard solutions were prepared at 1 mg mL^{-1} by dissolving a known amount of each compound in the suitable solvent (MeOH or water); they were then stored at −20 °C. Composite standard solutions were prepared by suitable dilution of stock solutions; a mix working standard solution containing all the 30 selected compounds was prepared at 0.05, 0.5, and 5 ng μL^{-1} based on single analyte instrumental response (see Supplementary Table S1 for detail). These solutions were stored at -20 °C while not in use and renewed weekly.

Oasis HLB cartridges (500 mg, 6 mL) were purchased from Waters (Milford, MA, USA), whereas GCB cartridges were prepared by manually packing 500 mg Supelclean ENVI-Carb bulk material into 6 mL polypropylene tubes between two polypropylene frits (Merck Life Science).

2.2. Designs of experiments for solid-phase extraction optimization

For the two sorbents, namely Oasis HLB and GCB, some parameters were selected from the literature and from previous experience of the group. Then, response surface using a Box-Behnken design (BBD) was used to optimize SPE conditions. Experiments were modelled and evaluated by DESIGN EXPERT V.13.0.4.0 (Stat-Ease, Inc., Minneapolis, MN, USA). Five replicates were carried out for the central point for pure error estimation.

For Oasis HLB sorbent, the numeric factors were: A) EDTA concentration (0, 0.025, and 0.05 mol L^{-1}) and B) pH value (3, 5, and 7) for sample loading and C) solvent volume (10, 15, and 20 mL) for sample elution (as reported in Table 1). Elution solvent was constituted by 5, 7.5, or 10 mL MeOH with 0.1% (*v/v*) FA followed by the same volume of MeOH with 0.1% (*v/v*) NH_3 interposed by 1.5 mL pure MeOH. The 17 resulting experiments were run in randomized order (see Supplementary Table S2).

Table 1. DoE parameters for Oasis HLB solid phase extraction

Factor	**Name**	**Type**	**Sub-type**	**Min (-1)**	**Mean (0)**	**Max (+1)**
A	EDTA conc (mol L^{-1})	Numeric	continuous	0	0.025	0.05
B	pH	Numeric	continuous	3	5	7
C	Eluent volume (mL)	Numeric	continuous	10	15	20

The DoE for GCB sorbent had 3 numerical variables, namely A) pH value (2, 4, and 6) for sample loading, B) GCB amount (200, 350, and 500 mg), and C) 10 mmol L^{-1} TMACl content (0, 50, and 100%) in the 20 mL (fixed value) of solvent elution mixture. Therefore, 0% TMACl corresponded to 20 mL CH_2Cl_2/MeOH 80:20 (*v*/*v*) 20 mmol L^{-1} TFA, 50% TMACl corresponded to 10 mL CH_2Cl_2/MeOH 80:20 (*v*/*v*) 20 mmol L^{-1} TFA followed by 10 mL CH_2Cl_2/MeOH, 80:20 (*v*/*v*) 10 mmol L^{-1} TMACl, and 100% corresponded to 20 mL CH_2Cl_2/MeOH 80:20 (*v*/*v*) 10 mmol L^{-1} TMACl. Moreover, a categorical variable was added, namely D) backflushing elution mode (see Table 2).

The 34 resulting experiments were run in randomized order (see Supplementary Table S3).

Table 2. DoE parameters for GCB solid phase extraction

Factor	**Name**	**Type**	**Sub-type**	**Min (-1)**	**Mean (0)**	**Max (+1)**
A	pH	Numeric	continuous	2	4	6
B	GCB (mg)	Numeric	continuous	200	350	500
C	TMACl (%)	Numeric	continuous	0	50	100
D	Backflushing elution	Categoric	nominal	yes		no

All the 51 experiments were carried out using 500 mL tap water spiked with 5, 50 or 500 ng L^{-1} of each of the 30 selected compounds, obtained by adding 50 µL of the mix standard solution (see Supplementary Table S1).

The two DoEs were evaluated using the mean recoveries of the 7 analyte groups. Responses were statistically evaluated by analysis of variance (ANOVA) at a confidence interval of 95% (p-value < 0.05). A desirability function provided by Design-Expert 13 was used.

2.2.1. Analyte recovery

Analyte recovery (RE%) was used for DoE evaluation. Tap water samples were spiked with standard mix before (set 1) and after (set 2) the extraction procedures. From the UHPLC-HRMS mass chromatograms, the exact *m/z* of $[M+H]^+$ of each analyte was extracted and the corresponding peak area was normalized with respect to the volumetric IS (caffeine-d9).

For each analyte, RE was assessed by the ratio between the normalized peak area of sample set 1 and sample set 2 according to the formula:

$$RE\% = \frac{A_{anal\ (set\ 1)} / A_{IS\ (set\ 1)}}{A_{anal\ (set\ 2)} / A_{IS\ (set\ 2)}} \times 100$$

2.3. River water samples

River water samples were collected in five different points of the area of Rome (Lazio, Italy): four points were chosen on the Tiber River and one point on the Aniene River, and the samples were named accordingly. Also, the position of the four WWTPs surrounding Rome was considered since the North, West, and South Plants release effluent to the Tiber River while the East Plant releases to the Aniene River (see Fig. 1).

The first sampling point was on the Tiber River, upstream of the City of Rome (Ponte del Grillo, TPG). The other four points were located within the Rome Municipality: on the Aniene River, before the confluence with the Tiber River (Ponte Salario, APS); on the Tiber River downstream the confluence with Aniene River and the North WWTP effluent release (Ponte Duca D'Aosta, TDA); on the Tiber River in the Center of the City (Ponte di Ferro, TPF); and on the Tiber River immediately downstream the City of Rome and the South WWTP effluent release (Tor di Valle, TTV).

Details on sampling and WWTP geographical locations are reported in Supplementary Table S4 and Table S5, respectively.

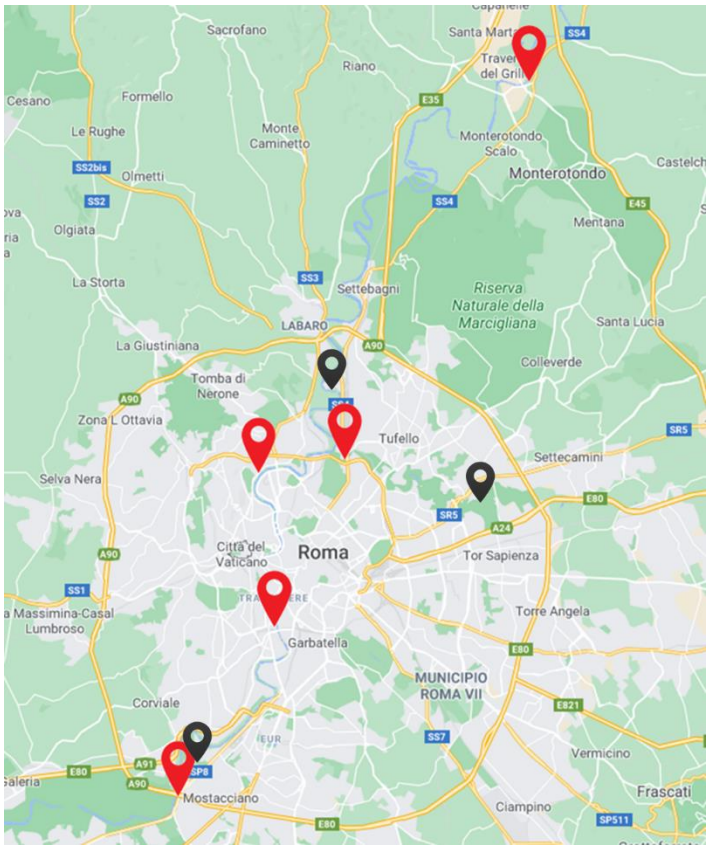

Fig. 1. Locations of the five sampling points (red pins) on the two rivers and of three wastewater treatment plants (black pins) in the City of Rome and surrounding.

2.4. Sample preparation and extraction

Aliquots of 500 mL of river water samples were analyzed on the same day in duplicate following the two different optimized SPE protocols. Samples were not subject to any filtration step before loading on the cartridge.

2.4.1. Protocol for Oasis HLB SPE cartridge

The Oasis HLB cartridge was previously washed with 5 mL of each elution solvent, i.e., MeOH with 0.1% (*v/v*) FA and MeOH with 0.1% (*v/v*) NH_3, and then it was conditioned sequentially with 5 mL MeOH and 10 mL of H_2O containing 0.025 mol L^{-1} EDTA and acidified with HCl to pH 4. A 500-mL river water sample was acidified with HCl to pH 4, added with EDTA to obtain 0.025 mol L^{-1} concentration, and loaded onto the cartridge with the aid of the vacuum at a flow rate of ca. 10 mL

min^{-1}. After washing with 5 mL of H_2O, the sorbent was left to dry for 15 min under vacuum. Compound elution was carried out by sequentially adding 8 mL of MeOH with 0.1% (*v/v*) FA, 1.5 of MeOH, and 8 mL of MeOH with 0.1% NH_3 (*v/v*).

2.4.2. Protocol for GCB SPE cartridge

Before use, the GCB cartridge was washed and conditioned sequentially with 10 mL of CH_2Cl_2/MeOH 80:20 (*v/v*) and 10 mL of MeOH, both containing 20 mmol L^{-1} TFA, 15 mL of 0.1 mol L^{-1} HCl, and 10 mL of ultrapure H_2O. The sample, constituted by 500 mL river water, was acidified to pH 4 with HCl and loaded onto the cartridge at ca. 20 mL min^{-1} with the aid of the vacuum. Then, the cartridge was washed with 10 mL of ultrapure H_2O acidified to pH 4. To eliminate the residual water from the cartridge, 1 mL of MeOH was passed slowly through the cartridge that was dried for 15 min under vacuum

A drilled cylindrical Teflon piston with one conically indented base and a Luer tip was inserted into the top of the cartridge until it reached the upper frit. Then the SPE cartridge was turned upside down for analyte elution in the back-flushing mode by 20 mL of CH_2Cl_2/MeOH 80:20 (*v/v*) containing 20 mmol L^{-1} TFA.

2.4.3. Final extract reconstitution

At the end of both procedures, to remove the solvents,eluates were placed in a water bath at 40 °C under a gentle nitrogen stream. To avoid possible compound degradation, solvent removal was stopped when the vial contained still ca. 50 µL of the eluate. The residue was added with 25 µL IS and reconstituted up to 250 µL to obtain a final composition H_2O/MeOH 70:30 (*v/v*).

The extract was centrifuged (MicroCL 21R centrifuge, Thermo Scientific) at 18,000 × g for 10 min to remove suspended particles; the supernatant was transferred to a clean vial for injection in the UHPLC-MS system.

2.5. UHPLC-HRMS analysis

The instrumentation consisted of a Vanquish UHPLC system equipped with a binary pump, a thermostated column compartment and an autosampler (kept at 14 °C), and coupled via a heated ESI source to a Q-Exactive hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The RP chromatographic column was a Hypersil Gold (150 × 2.1 mm i.d., 3 μm particle size) equipped with a Security guard Hypersil Gold (4 × 2.1 mm i.d., 5 μm particle size), both from Thermo Fisher Scientific. The column was maintained in the thermostated column compartment at 40 °C (still air option). Mobile phases were (A) H_2O, and (B) MeOH, both containing 0.1% (*v/v*) FA; flow-rate was 400 μL min^{-1}. The injection volume was 20 μL. The chromatographic gradient was as follows: after an isocratic step at 10% B for 4 min, B was linearly increased to 40% in 8 min and kept constant for 2 min; then B was brought to 70% in 6 min and kept constant for 5 min; finally, B was brought to 99% in 3 min, kept constant for 2 min for column washing, and then it was brought to the starting 10% in 2 min and the column let to equilibrate for 8 min (total run time 40 min).

Distinct UHPLC-HRMS runs were carried out for positive and negative ESI mode. Tune parameters were set as follows for positive and negative ion modes, respectively: spray voltage 3.3 and 2.5 kV; capillary temperature 320 and 280 °C; sheath gas 45 and 50 arbitrary units (au); auxiliary gas 15 and 10 au; spare gas 3.0 and 2.5 au; probe heater temperature 400 and 320 °C; S-lens RF level 55%.

For both ion modes, the full-scan MS acquisition range was 130–1000 *m/z*, the resolution was set to 35,000 (full width at half-maximum, FWHM, @200 *m/z*), and the automatic gain control (AGC) was set at 10^6, with a maximum ion injection time of 100 ms, and an isolation window width of 2 *m/z*. Data-dependent acquisition (DDA) mode was used to obtain the tandem mass spectra of the 5 most intense ions (for single scan cycle) by higher-energy collisional dissociation (HCD) at 40% normalized collision energy, with a resolution of 17,500 (FWHM), an AGC of 10^5, and a dynamic exclusion of 3 s. The mass spectrometer was externally calibrated every 48 hours, within a mass accuracy of 1 ppm, using the commercial Pierce positive and negative calibration solutions (Thermo

Fisher Scientific). Raw data files were acquired by Xcalibur software (version 3.1, Thermo Fisher Scientific).

For each sample, two technical replicates (UHPLC-HRMS runs) were performed, followed by two injections of a blank sample (MeOH/H_2O, 60:40 *v*/*v*) to allow column conditioning. Procedural blanks were also prepared for subtraction in data analysis.

2.6. Compound identification

Compounds were tentatively identified using Compound Discoverer v. 3.2 (Thermo Scientific) using a default workflow for untargeted environmental research with few modifications. Workflow scheme and parameters are reported in Supplementary Fig. S1 and Table S6, respectively. The identification was tentatively assigned based on the tandem mass spectrum match with the mass spectral database mzCloud (HighChem LLC, Slovakia).

3. Results and Discussion

3.1. Extraction optimization of contaminants from water samples

3.1.1. Target compound selection

The 30 compounds selected for SPE optimization (see Supplementary Table S1) belonged to different chemical classes since the aim was to optimize and compare two general SPE procedures for the untargeted analysis of CECs in environmental samples. Most of these compounds were pharmaceuticals, which are widespread surface water contaminants [5,17,22,24–30]: 19 were antibiotics of 4 different classes (fluoroquinolones, macrolides, penicillins, and sulfonamides); and 5 were steroid hormones (3 natural estrogens, 1 synthetic estrogen, and 1 synthetic glucocorticoid). The 2 antifungal imidazoles have veterinary/agricultural employment, and 1 (thiabendazole) has food preservative employment too. The last 4 compounds are common herbicides.

3.1.2 SPE protocol optimization

The hydrophilic/hydrophobic polymeric material, commercially available as Oasis HLB and other trademarks, is the most used in multi-residue methods, in particular for environmental applications, because of its sorption affinity for both polar and non-polar compounds [18–22,31].

However, this sorbent fails in recovering, for example, ionic compounds [31], and the short-chain and some emerging perfluoroalkyl substances (PFASs) [13], which are better recovered using anion exchange sorbents. On the other hand, GCB sorbent is reported in the literature as a suitable material for both aromatic and polar compounds [32,33], since it can establish diverse interactions with the analytes, including hydrophobic and electrostatic interactions for its graphene-like characteristics, as well as anion exchange capability; the GCB ability to retain anions by electrostatic interactions is provided by the chromene-like heterogeneities on its surface [33], making it an excellent sorbent for strong acidic compounds too [35–38].

For this reason, these two sorbents were selected and compared after condition optimization by a BBD. Some parameters, such as the sequence of Oasis HLB elution solvents containing FA and ammonia [22], as well as the CH_2Cl_2/MeOH (80:20, *v/v*) elution mixture for GCB [35–38], were selected from the literature. Also, EDTA addition to the sample is reported in the literature to improve the recovery of some antibiotics that could form complexes with metal ions [39]. For HLB DoE, a quadratic model was used for ANOVA, whereas for GCB DoE, for which also a categoric factor was included, a reduced cubic model was used. Results were evaluated by ANOVA considering the mean recoveries of the 30 selected compounds grouped in seven categories.

For Oasis HLB, the best conditions, as obtained from the 17 experiments, were sample acidified at pH 4.3 and containing 0.025 mol L^{-1} of EDTA, and an elution volume of 16 mL (see Supplementary Figs. S2-S3).

For GCB sorbent, the best conditions, as resulting from the 34 experiments, were 500 mg GCB amount, sample acidified to pH 3.9, elution solvent containing only TFA (0% ion-pair agent TMACl), and back-flushing elution mode (see Supplementary Figs. S4-S5). Indeed, reversing the GCB cartridge is a strategy very frequently adopted for reducing the solvent volume required for the elution

of the strongly retained analytes blocked on the top of the sorbent bed [40]. For GCB, EDTA addition was not considered since this chelating agent could be retained by the sorbent and saturate some active sites, as observed in preliminary experiments (data not shown).

Supplementary Table S7 reports the recoveries of the 30 analytes (evaluated as reported in 2.2.1. Analyte recovery subsection) obtained with the two SPE sorbents in the optimized conditions.

3.2. UHPLC-MS analysis

For RP chromatographic mobile phase, MeOH was preferred to ACN because in preliminary experiments better peak shape was obtained (data not shown). The HRMS acquisition followed a standard DDA for untargeted analysis. The resolution was set to 35,000 and 17,500 for MS and MS/MS acquisition, respectively, providing a mass accuracy below 2 ppm for most compounds. The acquisition was performed in both ion polarity modes but in distinct runs to maximize compound identification.

3.3. Compound identification

The untargeted analysis of river water samples provided 36190 and 15696 *m/z* features in positive and negative ionization mode, respectively. In positive ion mode, after filtering (removal of polyethylene glycols, polypropylene glycols, and other background contaminants), there were 1841 tandem mass spectra (999 unique *m/z* features, ranging between 125-1164 *m/z*), and 211 matching to mzCloud database were manually validated (see Supplementary Table S8). In negative ion mode, after filtering, 40 of the 307 tandem mass spectra were manually validated (see Supplementary Table S9). Only 10 compounds were identified in both ESI polarities, namely cannabidiolic acid, the 5 sartans candesartan, losartan, telmisartan, valsartan, and its metabolite, as well as 4,4'-dihydroxydiphenylsulfone, daidzein, kynurenic acid, and urocanic acid. Surprisingly, the short-chain perfluorobutane sulfonic acid (PFBS) was the only detected PFAS (see Supplementary Fig. S46). Some of the validated tandem mass spectra are reported in Supplementary Figs. S6-S46. All the

identifications reported in Supplementary Tables S8-S9 correspond to a confidence level 2a (probable structure) in the classification proposed by Schymanski *et al*. [15].

Among the detected *m/z* features, 8 were attributed to phthalates by mzCloud matching (from 81.4 to 99.5% best match); nevertheless, their identification was not manually validated, since in most cases their peak areas were not significantly different from procedural blank (see Supplementary Fig. S47), or in other cases, the protonated phthalic anhydride at *m/z* 149.0233 was the highest *m/z* fragment and an unambiguous assignment was not possible (see Supplementary Fig. S48).

Most of the identified compounds are therapeutic drugs, such as antibiotics, antihypertensive, and antidepressants, due to their ubiquitous presence in the environment [41] and therefore in the mass spectral database. For some of these (citalopram, clopidogrel, fipronil, sulfamethoxazole, tramadol, valsartan), the known TPs/metabolites were detected too. In agreement with previous reports, several drugs of abuse were identified [7], including cocaine and 4 of its metabolites.

3.4. Comparison between Oasis HLB and GCB in compound extraction

The identified compounds reported in Supplementary Tables S8-S9 were detected following both the SPE procedures. Also considering the whole detected *m/z* features, in terms of peak areas, the two sorbents gave comparable results for most compounds. For each tentatively identified compound, the peak areas of the experimental duplicates (2 technical replicates each) of all the samples extracted with a single SPE sorbent (20 runs) were averaged. The mean areas obtained with Oasis HLB sorbent were then compared with those obtained with GCB sorbent. Only peak area ratios>10 were considered significant. A better recovery was shown by Oasis HLB for compounds like gabapentin (GAB), mepivacaine (MEP), *O*-desmethylvenlafaxine (DVEN), venlafaxine (VEN), anisotropine (ANI), and clarithromycin (CLAR) (see Fig. 2a), whereas GCB performed better for compounds like veratrole (VER), edavarone (EDA), rimantadine (RIM), terbumeton (TBM), cannabidiolic acid (CAN), and ergocalciferol (ERG) (see Fig. 2b). Among these substances, CAN and ERG (better recovered by GCB) present the highest logP values, i.e., 6.6 and 7.4, respectively (source: Pubchem

https://pubchem.ncbi.nlm.nih.gov/), which very likely make the hydrophobic interactions predominant over the other retention mechanisms. On the contrary, GAB, better recovered by Oasis HLB, had the lowest logP value (<1).

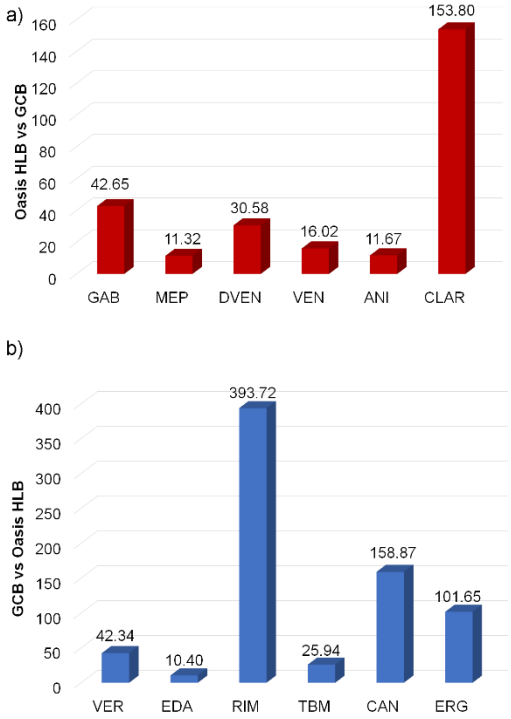

Fig. 2. Comparison between SPE sorbents. The mean peak area ratios show the better extraction efficiency for some analytes of: a) Oasis HLB; and b) GCB

3.5. Contamination in the different sample collection points

Different relative contamination levels for some analytes were observed in the samples collected from different points along the Tiber River (4 points) and the Aniene River (1 point) in the area of Rome. These differences are shown in Fig. 3a and 3b for samples extracted by Oasis HLB and GCB SPE, respectively. The analytes considered were those showing the largest difference between samples, namely 1,2-benzisothiazolin-3-one (BTZ), phenmetrazine (PHE), rimantadine (RIM), carbendazim (CBZ), chloridazon (CLZ), terbuthylazine (TBA), icaridin (ICA), minoxidil (MIN), propyphenazone (PRO), terbutryn (TBY), sulfadiazine (SDZ), ketoprofen (KET), lamotrigine (LAM), propranolol

(PRN), valsartan metabolite (VAL), methyl red (MRED), sotalol (SOT), citroflex 2 (CTX2), anisotropine methylbromide (ANI), abacavir (ABA), benzoylecgonine (BEC), climbazole (CLI), desmethylcitalopram (DCTP), acebutolol (ACE), ofloxacin (OFL), levofloxacin (LFX), alfuzosin (AFL), miconazole (MIC), irbesartan (IRB), flurandrenolide (FLU), raltegravir (RGV), Olmesartan (OLM), and tilmicosin (TIL).

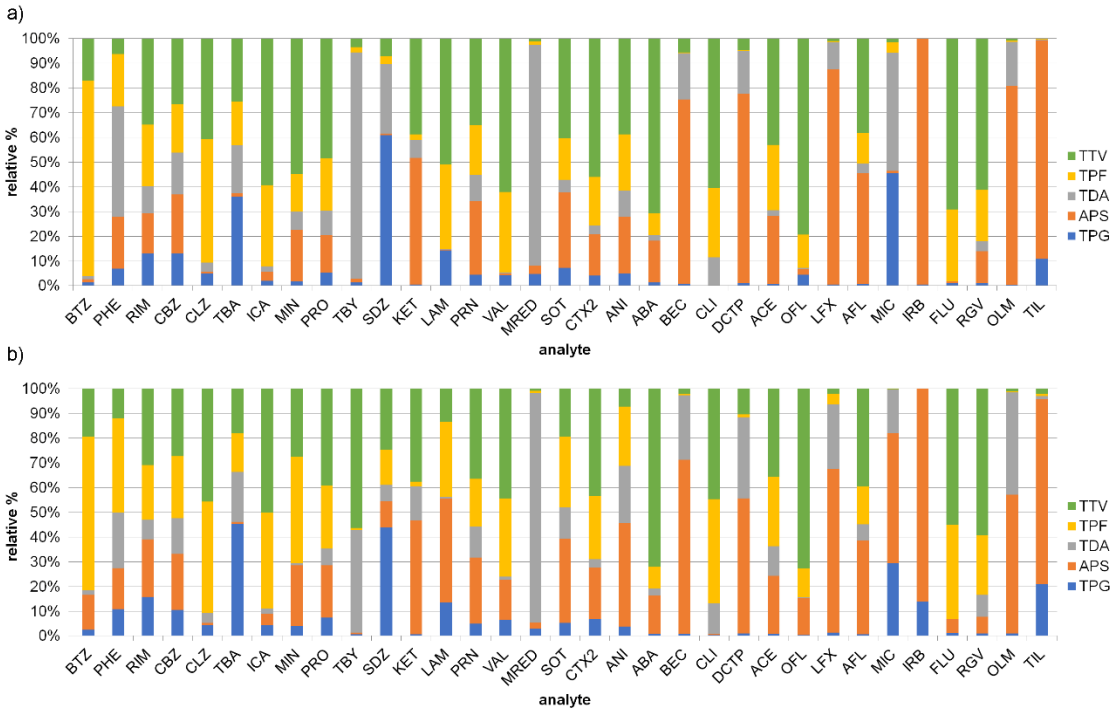

Fig. 3. River water samples extracted by a) Oasis HLB SPE and b) GCB. Comparison between samples collected in different points (TTV, TPF, TDA, APS, TPG) for the analytes showing the largest differences.

The samples collected from the Aniene River (APS) and the Tiber River downstream the City of Rome and the South Rome WWTP (TTV) were found to be the most contaminated by the selected analytes. For the sampling point TTV, the contamination is likely related to the impact of the City, mainly to the lack of CEC degradation during downstream transport, to incomplete removal before WWTP effluent release, and to illicit release or leakages. Indeed, for some CECs, the highest levels found at TTV were similar to those found in the samples collected in the City center at TPF (CLZ,

ICA, VAL, CLI). The samples collected from APS had similar contamination levels, likely more due to industrial than urban activities. As expected, the least contaminated samples were from the site upstream of the City of Rome (TPG).

4. Conclusions

In the last decades, the monitoring of CECs in the environment is generating an increasing interest by national authorities to preserve both humans and ecosystem health. The development and a more widespread availability than in the past of HRMS instrumentation are providing valuable support to this aim, because the HRMS technique allows detecting compounds whose presence is only suspected or even unknown, up to trace levels. This feature is fundamental, considering that at present tens of thousands of synthetic organic compounds are on the market and thus potentially dispersed in the environment.

In this work, 2 different sorbents for SPE, i.e., Oasis HLB and GCB, were first optimized and then applied for the extraction of CECs from river water samples. The 2 sorbents gave comparable results, even if each showed a better extraction efficiency towards some analytes. The untargeted analysis provided the tentative identification (level 2a of confidence) of 241 compounds. The analysis of samples collected in different points of the Tiber River and the Aniene River showed the impact of the City of Rome on the larger contamination for some compounds.

Appendix A

Supplementary Material: Tables S1-S7 and Figs. S1-S5

Supplementary Material: Tables S8-S9

Supplementary Material: Figs. S6-S48

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